Standard Operating Procedure For Determination of Mercury by Cold-Vapor Atomic Absorption

1.0 Location

Mercury determinations are performed in the Spectroscopy Laboratory, Room 305.

2.0 Purpose and Application

This method is applicable to drinking, surface and saline waters, domestic and industrial wastes to determine the amount of mercury in them. Detection limit for a 100-mL sample is 0.2 ug Hg/L.

3.0 Scope

3.1 Summary: This flameless atomic absorption procedure is a physical method based on the absorption of mercury vapor at 253.7 nm. Samples are digested in diluted potassium permanganate-potassium persulfate solutions and oxidized for two hours at 95° C. The mercury is reduced to the elemental state by stannous chloride and aerated from solution. The mercury vapor passes through a cell positioned in the light path of an atomic absorption spectrophotometer. Absorbance (peak height) is measured as a function of mercury concentration.

3.2 Interferences

3.2.1 Possible interference by sulfide is eliminated by the addition of potassium permanganate. Concentrations as high as 20 mg/L of sulfide as sodium sulfide do not interfere with the determination of mercury.

3.2.2 Interference from copper can occur when copper concentrations exceed 10 mg/L.

3.2.3 Sea waters, brines and industrial effluents high in chlorides require additional permanganate (as much as 25 ml). During the oxidation step, chlorides are converted to free chlorine which will also absorb radiation at 253 nm. Care must be taken to assure that free chlorine is absent before the mercury is reduced and swept into the cell. This may be accomplished by using an excess of hydroxylamine hydrochloride reagent (25 ml). In addition, the dead air space in the BOD bottle must be purged before the addition of stannous chloride. Both inorganic and organic mercury spikes have been quantitatively recovered from sea water using this technique.

3.2.4 Interference from certain volatile organic materials which will absorb at this wavelength is also possible.

3.2.5 In addition to inorganic forms of mercury, organic mercurials may also be present. These organo-mercury compounds will not respond to the cold vapor atomic absorption technique unless they are first broken down and converted to mercuric ions. Potassium permanganate oxidizes many of these compounds, but recent studies have shown that a number of organic mercurials, including phenyl mercuric acetate and methyl mercuric chloride, are only partially oxidized by this reagent. Potassium persulfate has been found to give approximately 100% recovery when used as the oxidant with these compounds. Therefore, a persulfate oxidation step following the addition of the permanganate has been included to insure those organo-mercury compounds, if present, will be oxidized to the mercuric ion before measurement. A heat step is required for methyl mercuric chloride when present in or spiked to a natural system. For distilled water the heat step is not necessary.

4.0 References

4.1 Methods for Chemical Analysis of Water and Wastes (EPA-600/4-79-020), Revision 3, May 1994, Method 245.1.

4.2 Standard Methods for the Examination of Water and Wastewater, 18th Edition, 1992, Method 3112 B, pg. 3-19 to 3-20.

5.0 Apparatus and Materials

5.1 Atomic Absorption Spectrophotometer, PE 3100.

5.2 Mercury Electrodeless Discharge Lamp (EDL).

5.3 Electrodeless Discharge Lamp (EDL) power supply.

5.4 Absorption cell.

5.5 Cell support.

5.6 Air pump (capable of delivering 1 liter of air per minute) and in-line regulator.

5.7 Glass aerator.

5.8 Connecting tubing (interconnects all components).

5.9 Reaction flasks (300 ml BOD bottles).

5.10 Drying tube (Desiccant).

5.11 Analytical balance.

5.12 Volumetric flasks.

5.13 Graduated cylinders.

5.14 Eppendorf pipet.

5.15 Waterbath and thermometer.

5.16 NOTE: All glassware should be acid soaked with a 30% nitric acid solution for at least two hours; then triple rinsed with distilled water and twice with ASTM Type II water or better.

6.0 Reagents (All reagents must be suitable for mercury analysis)

6.1 Deionized water.

6.2 Sulfuric Acid (concentrated, H_2SO_4), suitable for trace metal analysis.

6.3 Nitric Acid (concentrated, HNO_3), suitable for trace metal analysis.

6.4 5% Potassium permanganate solution (W/V): Dissolve 50g of potassium permanganate in water and dilute to 1 L.

6.5 5% Potassium persulfate solution (W/V): Dissolve 50 g of $K_2S_2O_8$ in water and dilute to 1 L.

6.6 10% Hydroxylamine hydrochloride solution (W/V): Dissolve 100 g of hydroxylamine hydrochloride in DI water and dilute to 1 L.

6.7 10% Stannous chloride solution (W/V): Dissolve 25 g of $SnCl_2$ in 25 mL of concentrated hydrochloric acid (HCl), with heating if necessary until clear, dilute to 250 ml. May be used as long as solution remains clear or for 1 month. On aging, this solution decomposes, if a suspension forms, discard and make fresh.

6.8 Magnesium perchlorate (used as a desiccant in the drying tube).

6.9 Stock Mercury Atomic Absorption Standard Solution, 1000 ppm.

6.10 Working Mercury Standard, 1 ppm (1ug/ml):

6.10.1 Add 100 ul of the 1000 ppm Stock Mercury Standard Solution to a 100 mL volumetric flask containing 90 mL of deionized water and 2 ml HNO_3.

6.10.2 Dilute to volume with deionized water and mix well.

6.10.3 Prepare fresh for each use.

6.11 Working Mercury Spike - prepare as in 6.10 using a different Stock Mercury Standard. The usual concentration for spiking is 1 ug/L Hg added.

6.12 12 % nitric acid solution.

7.0 Sample Handling and Preservation

7.1 Because of the extreme sensitivity of the analytical procedure and the presence of mercury in a laboratory environment, care must be taken to avoid extraneous contamination. Sampling devices, sample containers and plastic items should be determined to be free of mercury. The sample should not be exposed to any condition in the laboratory that may result in contamination from airborne mercury vapor. All glassware used in sample preparation must be acid rinsed, see sec. 5.16.

7.2 Samples should be preserved by acidification with nitric acid to a pH of 2 or lower immediately at time of collection or transported to the laboratory as soon as possible and acidified immediately upon receipt. Following acidification, the sample should be well mixed, held for 16 hours and then the pH verified to be less than 2. If the pH is not less than 2, the sample must be acidified again and allowed to set for 16 more hours and again the pH verified to be less than 2.

7.3 Samples are stored in the refrigerator.

7.4 Holding time - 28 days from time of collection.

8.0 Instrument operation

8.1 Perkin Elmer Atomic Absorption Spectrometer 3100 is used.

8.2 Install a mercury electrodeless discharge lamp. Connect to AA and EDL power supply.

8.3 Set wavelength to 253.7.

8.4 Set slit 0.7 and high.

8.5 Turn on AA power switch.

8.6 Press AA button.

8.7 Press Param Entry.

8.7.1 Set Energy 4.

8.7.2 Set Integration time 0.3 or less. Determine that the peak is broad and that highest concentration is being recorded.

8.7.3 Enter 1 Replicates.

8.7.4 Enter 1 Nonlinear.

8.7.5 Enter 1 Flame.

8.7.6 Enter no Std.

8.7.7 Enter no RSLP.

8.8 Turn on EDL power supply toggle switch. Set power to 4-5; whichever is right for the lamp you are using. Be sure lamp is on and is in the high energy mode (energy =50 or more). Energy level should be 60 or above when warmed up. Do not operate lamp in lower energy mode since this will damage the lamp.

8.9 Allow 45-60 minute warm-up time.

8.10 Install cell holder and cell in place of burner head assembly making sure beam passes through cell.

8.11 Attach pump and aerator to cell.

8.12 Press Energy

8.12.1 Be sure EDL current is set between 3.5 and 5 depending on lamp used.

8.12.2 Adjust wavelength to highest counts.

8.12.3 Adjust lamp position to highest counts with two knobs on top of lamp.

8.13 Press Cont

8.13.1 Adjust cell holder with three knobs for vertical, horizontal, and rotational alignment to lowest absorbance.

8.13.2 Auto-zero by pressing A/Z.

8.14 Press Energy

8.14.1 Press Gain

8.14.2 Energy should be about 60-70 and the counts about 100.

8.15 Press Param Entry. Press Print to print out parameter information.

8.16 Press Cont to read continuous absorbance with no printed record. Operator watches display and records highest absorbance on work list.

8.17 To read continuous absorbance with a printed record. Recommended.

8.17.1 To identify a sample: with Print off, enter number, press Print.

8.17.2 For each sample immediately before adding stannous chloride, with Print on, press Data, 0, Read to start printer. Listen for printer to start printing before adding stannous chloride to sample.

8.17.3 Printer continuously prints absorbance reading until stopped by pressing Read and CE.

8.17.4 Highest absorbance reading is usually recorded in 35-40 seconds.

8.17.5 Press Cont between samples to check zero.

9.0 Calibration and Standardization

9.1 Prepare one set of standards for each 20 samples. For each set measure 100 ml of deionized water into each of a series of five 300 ml BOD bottles, using a 100 ml graduated cylinder or dispenser.

9.2 Using an eppendorf pipette, transfer the following aliquot of the working mercury standard solution to each appropriately labeled BOD bottle according to the following table:

Aliquot Added (in ul)	Concentration (ug/L)
0	0.0
50	0.5
100	1.0
250	2.5
500	5.0

9.3 Mix each solution thoroughly by swirling the BOD bottle.

9.4 Treat each standard the same as the samples in the procedure section. The correlation coefficient should be 0.99 or better or the samples must be rerun. An Excel spreadsheet is used for all calculations. All data is printed and stored with sample data.

10.0 Procedure

WARNING: Because of the toxic nature of mercury vapor, care must be taken to avoid inhaling it. Therefore, the mercury vapor should be vented into an exhaust hood or passed through some absorbing media.

10.1 Using a 100 ml graduated cylinder, pour 100 ml of each sample into separate numbered 300 ml BOD bottles, record bottle numbers and log numbers on the worksheet.

10.2 Add 5 ml of concentrated H_2SO_4 and 2.5 ml of concentrated HNO_3 to each bottle and swirl to mix.

10.3 Add 15 ml of the 5% potassium permanganate solution to each bottle and swirl to mix.

10.4 Allow bottles to stand for 15 minutes. If the permanganate color disappears before 15 minutes, add additional $KMNO_4$ until the permanganate color persists for at least 15 minutes.

10.5 Add 8 ml of the 5% potassium persulfate solution to each bottle and swirl to mix. Stopper bottles and cap.

10.6 Place the bottles in a waterbath (maintained at a temperature of 95° C) and heat for 2 hours. Monitor temperature with a digital calibrated thermometer. Cover the bath. Standards do not need to be heated.

10.7 Remove the bottles from the water bath and allow them to cool to room temperature.

10.8 Add 6 ml of the 4% hydroxylamine hydrochloride solution or about 0.5 g of solid hydroxylamine hydrochloride to each bottle, mix and wait until the solution has decolorized (at least 30 seconds). Remove dead air space by blowing air into each bottle.

10.9 Analyze each standard and sample bottle individually. First add 5 ml of the 10% stannous chloride solution to the bottle and then immediately place the aerator inside the bottle. See Section 8.17.

10.10 Allow the sample to stand quietly without manual agitation.

10.11 As mercury is volatilized and carried into the absorption cell, absorbance will increase to a maximum within 30 seconds. Record this maximum value.

10.12 After maximum absorbance is obtained, remove the aerator from the bottle, rinse in a 12% nitric acid solution and place into a BOD bottle containing deionized water. Allow the instrument to return to baseline (minimum absorbance) and then proceed with the next standard or sample.

11.0 Calculations and Report

11.1 Construct a curve by plotting the absorbance readings of the standards against the concentration of the standards in micrograms (ug) of mercury. The correlation coefficient should be 0.99 or better or the samples must be rerun.

11.2 Compare each sample's maximum absorbance value to the standard curve.

11.3 Calculate the mercury concentration in the sample by the formula:

$$\text{ug Hg/L} = \frac{(\text{ug Hg in aliquot of sample})\ (1000)}{\text{volume of aliquot in mL}}$$

11.4 As an alternative to the above calculation, a spreadsheet employing linear regression may be used to determine the mercury concentration of the samples.

11.5 An Excel spreadsheet is used for all calculations and printed and saved.

11.6 Report mercury concentration as <0.2 ug/L if the concentration of the sample is below 0.2 ug/L. If the concentration of the sample is greater then 0.2 ug/L, the result must be verified by reanalyzing the sample in duplicate and reporting that number.

12.0 Quality Control

12.1 An LRB (reagent blank) and calibration blank shall be analyzed in each run.

12.2 Spike and duplicate 10% of all samples analyzed with a minimum of one spike and one duplicate per run. Analyze a spiked blank (lab fortified blank) in each run.

12.3 An instrument performance check solution (made from same source as standards) shall be run with each analysis for every 10 samples. Recovered mercury concentration of this sample must be within acceptable limits of 90-110% or samples must be rerun. A quality control sample obtained from a source external to the laboratory and different from the standards is run whenever a new standard source is used or whenever needed to verify calibration standards and acceptable instrument performance.

12.4 Initial Demonstration of performance.

12.4.1 A mercury MDL should be established using reagent water (blank) fortified at a concentration of two to five times the estimated detection limit. To determine MDL values, take seven replicate aliquots of the fortified reagent water and process through the entire analytical method. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

$$MDL = (t) \times (S)$$

where: t = value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom is, t = 3.14 for seven replicates.

S = standard deviation of the replicate analysis.

12.4.2 An MDL should be determined yearly or whenever a significant change in background or instrument response is expected and documented.

12.4.3 Linear calibration ranges: The upper limit of the linear calibration range should be established for mercury by determining the signal responses from a minimum of three different concentration standards, one of which is close to the upper limit of the linear range. Linear calibration ranges should be determined every six months or whenever a significant change in instrument response is observed.

12.5 Assessing Laboratory Performance: Reagent and fortified blanks

12.5.1 The laboratory must analyze at least one LRB with each set of samples. LRB data are used to assess contamination from the laboratory environment and to characterize spectral background from the reagents used in sample preparation. If a mercury value in an LRB exceeds its determined MDL, then laboratory or reagent contamination is suspected. Any determined source of contamination should be eliminated and the samples reanalyzed.

12.5.2 One LFB (spiked blank), with a usual concentration of 1 ug/L added, must be analyzed with each batch of samples. Calculate accuracy as percent recovery. If recovery of mercury falls outside control limits (see section 12.5.3), the method is judged out of control. The source of the problem should be identified and resolved before continuing analyses.

12.5.3 Performance should be assessed against recovery limits of 85-115%.

12.6 Assessing analyte recovery: Laboratory fortified sample matrix (spiked samples)

12.6.1 Spike 10% of samples or at least one per run. Select a representative water sample of the type of samples being analyzed which has a low mercury background. It is recommended that this sample be analyzed prior to fortification. The spike amount should be 20% to 50% higher than the analyzed value. Over time, samples from all routine sample sources should be fortified.

12.6.2 Calculate the percent recovery, corrected for background concentrations measured in the unfortified sample, and compare these values to the control limits established in Sec. 12.5.3 for the analyses of LFBs. A recovery calculation is not required if the concentration of the analyte added is less than 10% of the sample background concentration. Percent recovery may be calculated in units appropriate to the matrix, using the following equation:

$$R = \frac{Cs - C}{} \times 100$$

s

where, R = percent recovery
Cs = fortified sample concentration
C = sample background concentration
s = concentration equivalent of fortifier added to water sample.

12.6.3 If mercury recovery falls outside the designated range, and the laboratory performance is shown to be in control (see sec 12.5), the recovery problem encountered with the spiked water sample is judged to be matrix related, not system related. The result for mercury in the unspiked sample must be labeled to inform the data user that the results are suspect due to matrix effects.

13.0 Documentation

13.1 All information and values are recorded on a work list and spreadsheet printout.

13.2 Parameter information is saved. See section 8.15.

13.3 Printouts of data from instrument for standards and samples are saved. See section 8.17.

13.4 Spreadsheet calculations are printed and saved.

13.5 LIMS printout records are also saved.

14.0 Records

All print outs pertaining to Safe Drinking Water Act samples including work lists, parameter information, computer print outs and LIMS printouts are stored in Mercury #16 logbook located in the bookcase in room 305. See section 13.0 for complete documentation..

15.0 Safety

15.1 The toxicity and carcinogenicity of each reagent used in this method have not been fully established. Therefore, each chemical should be regarded as a potential health hazard and exposure to these compounds should be minimized by good laboratory practices.

15.2 Mercury compounds are highly toxic if swallowed, inhaled, or absorbed through the skin. Analyses should be conducted in a laboratory exhaust hood or in another

suitable exhaust system. The analyst should use chemical resistant gloves when handling concentrated mercury standards.

15.3 The acidification of samples containing reactive materials may result in the release of toxic gases, such as cyanides or sulfides. Acidification of samples should be done in a fume hood.

15.4 All personnel handling environmental samples known to contain or to have been in contact with human waste should be immunized against known disease causative agents.